Impact of ultrasound settings on lung vertical artefacts: an observational study in mechanically ventilated patients

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Shareable abstract (@ERSpublications)
Ultrasound settings influence the number of vertical artefacts. To minimise the impact of pre-sets on vertical artefacts, adjust gain, power and dynamic range to have a hyperechoic pleura and turn off filtering. https://bit.ly/46GaThh

Cite this article as: Leote J, Gonçalves A, Fonseca J, *et al*. Impact of ultrasound settings on lung vertical artefacts: an observational study in mechanically ventilated patients. *ERJ Open Res* 2025; 11: 00483-2024 [DOI: 10.1183/23120541.00483-2024].

Received: 24 May 2024
Accepted: 29 July 2024

Abstract

Introduction The number of vertical artefacts (VAs) in lung ultrasound (LUS) impacts patients' clinical management. This study aimed to demonstrate the influence of ultrasound settings on the number of VAs in patients under invasive mechanical ventilation (IMV).

Methods Patients under IMV were recruited for LUS, including three breathing cycles with a motionless curvilinear probe on the thoracic region with the most VAs. Three experts in LUS were asked about the number of VAs at random, and blinded after altering the settings for a total of 20 test recordings per patient. The correlation between expert classifications was tested after grading the classifications. The number of VAs across clinicians was compared between baseline recordings and test condition recordings to determine statistical differences.

Results 29 patients were enrolled with a median Sequential Organ Failure Assessment score of 6 (interquartile range (IQR) 3). IMV was mainly due to stroke (n=10) and pneumonia (n=6). LUS was made between days 1 and 6 (IQR). Baseline recordings showed a median of 2±2 VAs in inspiration and a median of 1±2 in expiration from a total of 3636 expert classifications, with a strong agreement within patients. A probe frequency of 8 MHz, artefact filtering, speckle reduction and frame average reduced the median VA number by one. A power of −20 dB and dynamic range of 32 dB abolished the VAs. A gain above 90% increased the median number of VAs by one.

Conclusion In this *in vivo* study, the LUS settings influenced the VA number in IMV patients, after controlling for physiological and operator confounders.

Introduction

Vertical artefacts (VAs) seen in lung ultrasound (LUS) are nonspecific image features that can be detected in cardiogenic and noncardiogenic pulmonary oedema, such as acute respiratory distress (ARDS) [1–3]. These artefacts, may also be called B-lines, a nomenclature adapted from the radiological chest radiography Kerley B-lines (or interlobar septal lines) reported to occur with increased pulmonary capillary wedge pressure [4].

The VAs originate from the diminished impedance between thoracic *s.c.* tissue and aerated lung within the presence of interstitial oedema. The energy pulse emitted by the ultrasound (US) is transmitted through a pathway according to the medium's impedance (*i.e.* channel), and is therefore reflected towards the probe or to the interstitial parenchyma, resulting in echo wave traps [5, 6]. Echo wave scattering of different intensity when trapped inside channels of variable shape, volume, content and contact size with pleura, promotes changes in VA morphology across diseases [7].

LUS is a relevant adjunct tool for monitoring lung diseases, to such an extent that the European Respiratory Society [8] and international consensus [9] reviewed and reported standards, considering the new scientific developments, to highlight the use of LUS in clinical practice. One learning point from LUS daily practice was the comprehensive understanding that US system settings influence the VAs and data interpretation. Some US settings were already known to influence the morphology of VAs (or B-lines) [1, 10–13], but after the SARS-CoV-2 pandemic, a more multidisciplinary approach (*i.e.* translational, bioengineering research) was driven to improve LUS reproducibility [8, 9].

Translational studies searched for optimal US system settings to LUS avoiding a negative impact on VA appearance. The *in vivo* reports focused more on settings that easily have a profound and discernible impact on the number of VAs (*i.e.* probe type, focus position and tissue compound imaging). However, patient movements, thorax or lung compliance, the amount of volume entering into the lungs and particularly the probe movements were not controlled. The *ex vivo* and *in silico* reports used a more complex methodology to perceive and quantify any decrease in VA signal intensity, which, to some extent, translates to *in vivo* experiments (*i.e.* probe centre frequency, power, gain and frame averaging). Therefore, the appearance of VAs differs depending on the US pulse centre frequency, bandwidth, the shape of the probe (*i.e.* angle of incidence), focal zone position and imaging depth range, in addition to the US system power, gain, dynamic range and advanced post-processing tools [10–14]. Nevertheless, technical, operator and disease confounders should influence the interpretation of VAs and their clinical impact is ongoing [15, 16]. Therefore, it would be desirable to produce comprehensive clinical studies evaluating and providing an understanding of the influence of technical factors on VAs. By performing lung US with a static probe and by controlling the amount of lung aeration, US *in vivo* recordings would be standardised as much as possible. In addition, the knowledge about thoracic respiratory mechanics would provide valuable information for interpreting the VA presumable aetiology and its implications for clinical syndrome.

In the present study, patients under passive invasive mechanical ventilation (IMV) were recruited to test US setting conditions. The aim was to demonstrate comprehensively how the settings available in a conventional US system may routinely influence the morphology and number of VAs.

Methods

Patient enrolment and ethical approval

Patients aged 18 years old or older and under passive IMV were prospectively recruited between June and August 2023. The following exclusion criteria were used: patients with a medical history of pulmonary fibrosis or pulmonary cystic fibrosis, lung tumours, pulmonary embolism, asthma, COPD, bronchopleural fistula, tracheostomy, lung transplant and pregnancy. Afterwards, patient clinical data such as clinical symptoms, signs and reasoning for IMV and intensive care unit (ICU) admission was reviewed. Patient disease severity was evaluated by Acute Physiology And Chronic Health Evaluation II (APACHE II) and by Sequential Organ Failure Assessment (SOFA) on admission and daily. The patient's demographic and clinical data were anonymised by attributing a numerical reference. Future management of data was done using such reference, and the corresponding matrix was kept hidden from all of the investigators involved (by author FG). The Ethics Committee of the Hospital Garcia de Orta EPE (Almada, Portugal) approved the study protocol (number 49/2023). Subjects were asked to sign a digital informed consent before IMV or after IMV weaning. In the case of cognitive impairment in both periods, informed consent was asked to the patient's legal representative. If not available, the Ethical Committee was contacted and the necessity for informed consent was waived.

Patients' clinical bedside data before US examination

The level of alertness and patient behaviour was evaluated by the Richmond Agitation Sedation Scale and aimed at a score between −2 and −3 with the use of propofol and remifentanil infusion. The following respiratory parameters were evaluated from the mechanical ventilator: fraction of inspired oxygen (F_{IO_2}), arterial blood oxygen saturation (S_{aO_2} %), tidal volume (mL), plateau pressure (cmH_2O), positive end-expiratory pressure (PEEP, cmH_2O) and days of ventilation. During LUS evaluation, patients were passively ventilated under pressure-regulated and volume-controlled mode without spontaneous triggers. The LUS assessment was done 15 min after adjusting IMV for low tidal volume (6–7 $mL \cdot kg^{-1}$) and stable levels of arterial partial pressure of oxygen (65–70 mmHg) and carbon dioxide (35–40 mmHg). Cardiac performance was also recorded on the same day as LUS using transthoracic echocardiography (by an examiner accredited by the European Association of Cardiovascular Imaging). The cardiac index ($L \cdot min^{-1} \cdot m^{-2}$) and the tissue doppler-derived E wave/e′ ratio were calculated. If necessary, the mean arterial pressure was kept above 65 mmHg using norepinephrine. Patients' diuresis was ensured to be within the normal range (>1 $mL \cdot kg^{-1} \cdot h^{-1}$) over the last 48 h. The daily and cumulative fluid balance was also recorded.

Experimental and imaging setup

The US recording of all patients was done by one author (JL), who was instructed to review the patient bedside data for changes. The examiner was also blind to clinical symptoms, admission diagnosis and disease severity. The patients enrolled in the study were in a semi-recumbent position to allow conventional LUS of eight regions in each part of the thorax. Then, the region with the most B-lines and easiest to set up the LUS examination was selected. A 10-cm high iron structure, consisting of a metal base with a flexible aluminium arm at the top, was used to fixate the probe over the patient's thorax. To warrant a motionless probe during recordings, a built-in cell phone (Huawei p40 pro, China) gyroscope was applied to the iron structure using the Physics Toolbox application (Vierya Software, Washington, USA) [12]. The probe was placed with a side marker towards the cranial part of the patient, perpendicular to the ribs, starting from a conventional window using the intercostal space as a reference (*i.e.* "bat sign"). Three-dimensional movements were done to record the most intense (*i.e.* hyperechoic) signal of the VAs [8, 9].

For US image acquisition, the MicrUs EXT-1H beamformer (Telemed, Vilnius, Lithuania) was used to produce echo waves through a micro curvilinear probe with a 20-mm radius and 5-mm lateral dimension, system frequency of 4 MHz, field of view of 46 degrees or 97 mm, with 64 piezoelectric elements (code MC8-4R20S-3, Telemed). The US signal was recorded with a 40 MHz bandwidth sampling frequency and frame rate up to 120 Hz and the signal was converted to digital using an 8-bit converter before being exported in an anonymised DICOM format.

US images were recorded using the following settings: mechanical index of 0.5; depth range between 80–100 mm (at least 60 mm below the pleura); one focal depth (on the pleura) the power of −10 dB; gain of 80%; equal level of time gain compensation across depth, standard line density, a dynamic range of 60 dB, without tissue equalisation or optional post-processing tools (supplementary table 1 and supplementary material). DICOM recordings were made with 5-s duration, which included at least two respiratory cycles. This setup was reported previously [12]. Inspiration and expiration were identified through M-mode, which ran simultaneously with the B-mode (supplementary figure 1 and supplementary material). An M-mode marker was placed on one side of the echo window near the rib and was used a sweep of 6.68 s with a 30-mm depth, including the pleura.

Number of VAs, data reduction and statistical analysis

In each patient, a thoracic region was screened by US for baseline recordings using the pre-set defined in supplementary table 1. In each patient, an individual setting was changed from the baseline to test recording (supplementary table 1) and a new test US cine loop was made. The US baseline and test recordings (20 US cine loops) were named and anonymised by two authors (AG and JF). The key showing the correspondence between the clip and the US parameter tested was kept only by them. Afterwards, the US recordings were uploaded in one critical care department computer after converting the DICOM files to mp4 format. Consequently, three out of five experts in LUS of the ICU were asked to answer a query for each US clip with the following questions regarding each clip. Question 1: "Do you see any VAs?"; question 2: "How many do you see during inspiration?"; question 3: "How many do you see during expiration?"; and question 4: "Do you see any pleural irregularities associated with the VA?". For each question, the following answers were available: "yes or no" for questions 1 and 4; and 1, 2, 3, 4, 5, 6 or >7 VAs for questions 2 and 3. The questionnaire was conducted electronically using Google forms (Google HQ, California, USA) referring to the anonymised US cine loops stored on the department's computer. VAs were defined as triangular artefacts with a downstream shape, hyperechoic with homogenous and clearly distinguishable intensity from the background, arising from pleura to at least three-quarters of the screen without a significant decrease in signal intensity, which moved with lung sliding. The pleura was evaluated for fragments and irregularities within the pleura line.

For analytical purposes, descriptive statistics were used for the patients' demographic, bedside and clinical examination variables. LUS cine loops were reviewed by three experts for the number of VAs. The number of VAs during inspiration and expiration were extracted from the answers from the experts. The presence of outliers was considered as one number of VAs made by one of the clinicians, with more than 3×SD from the mean VA number. In this case, a fourth expert was asked to review the cine loop and quantify the VA and their evaluation replaced the one discrepant. Before performing statistical tests, we ensured that the extracted data complied with all the required assumptions. Correlation between the experts' number of B-lines was achieved using Kendall's correlation coefficient after grading the following intervals: grade 0, one to two VAs; grade I, three to six VAs; and grade II, more than six VAs. The influence of US parameter (*i.e.* group) with different conditions (*i.e.* test recordings) on the number of VAs observed in baseline recordings was tested for significant difference. A Friedman test was run to determine if there

were differences in the number of VAs during the test recordings. In addition, pairwise comparisons were performed with a Bonferroni correction for multiple comparisons using an adjusted significance level according to the number of conditions. Overall, a p-value <0.05 was considered to indicate statistical significance. This statistical analysis was performed using SPSS (version 27, IBM, Chicago, USA).

Results

Patient demographics

A total of 29 patients were enrolled during the study period and the demographic details and bedside clinical data are shown in table 1. The patients showed a mean APACHE II score of 18 points, a SOFA score of six points on the first 24 h and a mean delta SOFA of two points during hospital stay. The patients' need for IMV was classified using the primary aetiology responsible for the respiratory failure. Among the neurological diseases, the most common cause was stroke (six patients) and spontaneous subarachnoid haemorrhage (four patients). Whereas within the respiratory diseases, the most common cause was interstitial pneumonia in four patients followed by ARDS in two patients. Other aetiologies included refractory septic shock in six patients and two patients with post-cardiac arrest (supplementary table 2 and supplementary material). The LUS examination in half of the patients was done on the third day after ICU admission. Taking into account the patients' diseases, clinical, laboratory and imaging data, the VA aetiologies were considered as pulmonary congestion (seven patients), infectious (eight patients), cardiogenic (two patients), miscellaneous (three patients) and nonspecific in nine patients (supplementary table 2 and supplementary material).

LUS and the number of VAs

The 29 LUS tests were performed at an anterior or lateral part of the lower thorax. The dataset included US baseline recordings (29 cine loops) and US test recordings (574 cine loops). The US dataset of each patient comprised 20 cine loops in three patients and 21 cine loops in the remaining patients. An example of LUS is shown in figure 1. Six test US cine loops were not done in three patients and were considered as missing data (testing of speckle reduction, level 5 and level 8). In addition, a total of 19 cineloops were dismissed due to probe movement related to the operator (11 cine loops) and with the ICU environment (eight cine loops).

TABLE 1 Demographic, disease severity scores and physiological details of the patients (n=29) recruited for lung ultrasound evaluation

Age, years	57.8±17.8
Gender female/male, n	10/19
BMI, kg·m^{-2}	27.1±6.4
Height, cm	168.8±10.8
APACHE II, score	17.9±6.4
SOFA, score, median (IQA)	6 (3)
Delta SOFA, score, median (IQA)	2 (2)
IMV aetiology, neurological/respiratory/others, n	17/6/6
IMV, days, median (IQA)	3 (3)
F_{IO_2}, fraction, median (IQA)	0.35 (0.10)
POS, fraction, median (IQA)	0.98 (0.03)
Tidal volume, mL	442±64
Plateau pressure, cmH_2O	16.5±3.1
PEEP, cmH_2O, median (IQA)	5 (1)
Cardiac index, L·min^{-1}·m^{-2}, median (IQA)	3.3 (0.8)
E/e′, fraction, median (IQA)	8 (6)
Norepinephrine[#], µg·kg^{-1}·min^{-1}	0.5±0.3
Peripheral oedema[¶], n	15
Daily fluid balance, mL, median (IQA)	500 (1552)
Total fluid balance, mL, median (IQA)	3094 (3101)

Data are presented as mean±SD unless otherwise specified. APACHE II: Acute Physiology and Chronic Health Evaluation; BMI: body mass index; F_{IO_2}: fraction of inspired oxygen; IMV: invasive mechanical ventilation; IQA: interquartile amplitude; PEEP: positive end-expiratory pressure; POS: peripheral oxygen saturation; SOFA: Sequential Organ Failure Assessment; E/e′: tissue doppler-derived E wave/e′ ratio. [#]: Only seven patients were under vasopressor. [¶]: Peripheral oedema was considered present if pitting oedema with a depth of 4 mm and rebound less than 15 s on both legs.

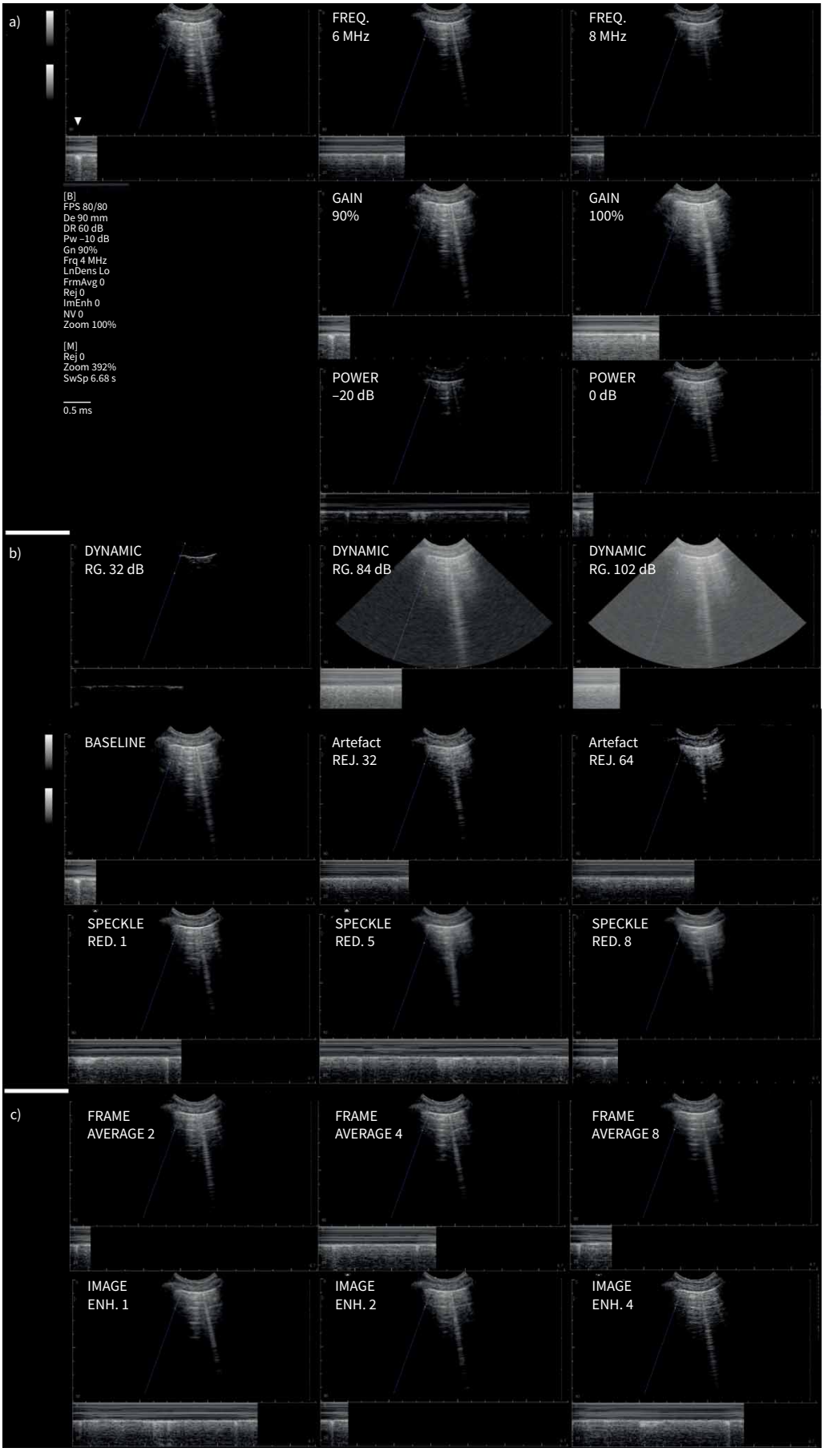
a)
[B]
FPS 80/80
De 90 mm
DR 60 dB
Pw –10 dB
Gn 90%
Frq 4 MHz
LnDens Lo
FrmAvg 0
Rej 0
ImEnh 0
NV 0
Zoom 100%
[M]
Rej 0
Zoom 392%
SwSp 6.68 s
0.5 ms
FREQ. 6 MHz
FREQ. 8 MHz
GAIN 90%
GAIN 100%
POWER –20 dB
POWER 0 dB
b)
DYNAMIC RG. 32 dB
DYNAMIC RG. 84 dB
DYNAMIC RG. 102 dB
BASELINE
Artefact REJ. 32
Artefact REJ. 64
SPECKLE RED. 1
SPECKLE RED. 5
SPECKLE RED. 8
c)
FRAME AVERAGE 2
FRAME AVERAGE 4
FRAME AVERAGE 8
IMAGE ENH. 1
IMAGE ENH. 2
IMAGE ENH. 4

FIGURE 1 Illustrative example of patient with interstitial pneumonia and focal abnormalities on lung ultrasound recordings (patient 6; supplementary table 2 and supplementary material). M-mode was also used to identify lung sliding and ensure that the vertical artefact showed was the same in every frame. Note a pleura irregularity in M-mode and the frame time (arrowhead). The baseline frame is located in the top left corner allowing a comparison between test conditions frames. The ultrasound settings were grouped by a) frequency and signal intensity, b) artefact filtering and c) image enhancement. Note the negative impact on vertical artefact signal intensity and length by frequency (8 MHz), power (−20 dB), dynamic range (32 dB), artefact rejection (64 elements) and speckle reduction (level 8).

The questionnaires generated a pool of 3636 answers for the VA number, including inspiration (n=1818) and expiration (n=1818) after removing the missing data. Between 60 and 126 answers were obtained during inspiration and expiration for each patient. Although the queries were answered by three clinicians, 80 answers (2.2%) were considered extreme outliers and, therefore, a fourth expert re-evaluated the LUS cine loops and re-classified them accordingly. However, there remained 82 answers (out of 3636, 2.3%) in the data pool with a VA number of more than 1.5×SD in comparison with the mean VA for a specific US cine loop. Nevertheless, on baseline recordings, there was a strong, positive association between the number of VA grades given by the three experts (*i.e.* experts A, B and C), which was statistically significant (Kendall's correlation coefficient), between A and B (Tau-b=0.611, p=0.001), between B and C (Tau-b=0.604, p=0.004) and between C and A (Tau-b=0.698, p=0.0005).

The median number of VAs for the baseline recordings and test recordings after varying US conditions of different parameters is shown in figure 2 and table 2. Pleura irregularities were found in 10 patients and 7 presented with infectious pneumonia (supplementary table 2 and supplementary material). The median number of VAs across the population in baseline recordings was one with an interquartile range of two B-lines. On the baseline, more VAs were found during inspiration (table 2). The US parameters tested significantly influenced the number of VAs, with the exception of the image enhancement and the scarce influence of the speckle reduction. The Friedman test results for each US parameter and tested conditions are shown in supplementary table 3.

The frequency of 8 MHz diminished the median number of VAs by one during all respiratory cycle. In fact, higher probe frequencies decrease the VA length at depth, as shown in figure 1a. Similarly, a gain of 100% increased VA by one in comparison with baseline recording by enlarging VA width (figure 1a). The decrease of US power to −20 dB and the dynamic range for 32 dB abolished the VA (table 2). Accordingly, these US settings, when used, cleared the VAs, as shown in figure 1a. High values of dynamic range (84 and 102 dB) did not consistently raise the median of the number of VAs during inspiration and expiration (figure 2a). Nevertheless, the value of 82 dB showed a tendency for significant difference during the whole respiratory cycle VA number (table 2). Additionally, the VA width increased, whereas image contrast decreased (figure 1a).

Regarding artefact reduction, when this filter was used in 64 elements of the probe, the median number of VAs was reduced by one in comparison with the baseline, which is evident in figure 1b. Only the tested level 8 of the speckle reduction diminished the clinicians' VA number (figure 2c). Of note, a progressive decrease in signal intensity from level 1 to level 8 (*versus* off) on the first one-third of the VAs below the pleura (figure 1b) was seen. On the other hand, averaging frames only affected the median number of VAs when using level 8 (*versus* off). Such findings were not reproducible by the motionless frames and a representative case is shown in figure 1c. In addition, an illustrative example of a patient with ARDS (patient 11; supplementary table 2 and supplementary material) is shown in video 1 to demonstrate the influence of LUS settings on the VA number during clinical practice (supplementary video 1 and supplementary material).

Discussion

This work demonstrated the influence of US settings on the number of VAs during inspiration and expiration in patients under passive IMV after controlling for operator and relevant physiological confounders on VA genesis. To avoid a negative US system influence on the VA number, our findings show that the US pre-sets should use a lower probe frequency (*i.e.* 4 MHz), gain and power adjusted to a value near the available upper limit (*i.e.* gain 90%, power −10 dB), an intermediate value of dynamic range (*i.e.* between 60 and 84 dB) and without any post-processing tool such as artefact reduction, speckle reduction, frame averaging or image enhancement.

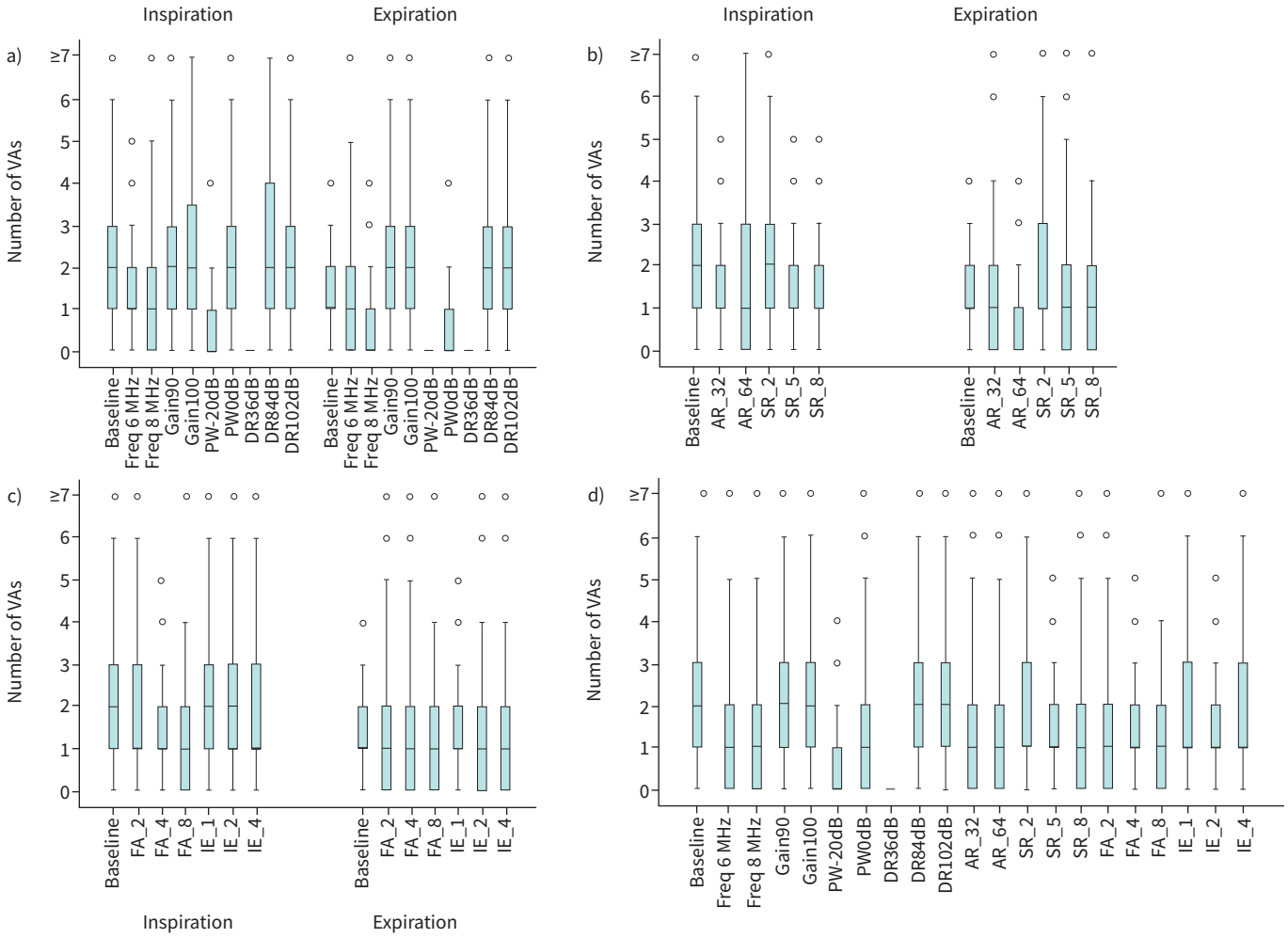

FIGURE 2 Boxplot graphs showing descriptive statistics regarding the number of vertical artefacts (VAs) after three experts reviewed the lung ultrasound baseline recordings and test recordings of 29 patients during inspiration and expiration. The ultrasound settings were grouped by a) frequency and signal intensity, b) artefact filtering and c) image enhancement. More VAs were identified in inspiration than in expiration. d) Statistics of all respiratory cycle VA numbers are shown. Dynamic range (84 dB) and speckle reduction (level 8) showed a tendency for a difference in comparison with baseline recordings but was not consistent in isolated inspiration nor expiration (table 2). AR: artefact rejection; DR: dynamic range; FA: frame average; Freq: frequency; IE: image enhancement; PW: power; SR: speckle reduction. Circles show VAs with 1.5×SD greater than the mean.

Patient enrolment, VA detection and passive mechanical ventilation

The definition of B-lines was recently challenged for VAs due to the influence of US settings on B-line morphology [9]. BUDA *et al.* [16] demonstrated that if VA length decreases after changing the probe frequency from 2 MHz to 6 MHz, VA genesis was more likely to be pulmonary fibrosis than cardiogenic pulmonary oedema. In our cohort, the aetiology of the VA was extravascular lung water and inflammatory pulmonary oedema in half of the patients, cardiogenic oedema in two patients and nonspecific oedema in the remaining patients. Biophysical knowledge about VA origin supports the application of quantitative tools to estimate surface lung roughness [17, 18], which would help clinicians to interpret the pathophysiological background of the VA. Therefore, VAs with distinctive origins may behave differently to the change of US settings. In our study, clinicians were instructed to identify VAs resembling the "comet tails" described in patients with increased extravascular lung water [1], possibly excluding other VAs from the described results. Nonetheless, until the application of quantitative tools in clinical routine, a syndromic whole thorax examination, as well as the assessment of the pleura, VA thoracic distribution (*i.e.* spared regions and gravity dependence) remain the LUS point of reference [9].

Lung B-lines, like other VAs, are generated in an altered air-to-water content ratio in the alveoli [6]. In our study, the US recordings were done in passive patients under invasive closed circuit, volume-controlled

TABLE 2 Influence of ultrasound parameters on the number of vertical artefacts from 29 mechanical ventilated patients; data were extracted from the classification undertaken by three experts in each phase of the respiratory cycle

Ultrasound parameters, unit	Respiratory cycle		Inspiration		Expiration		All	
	Test value (*versus* baseline value)		Median (IQA)	p-value[¶]	Median (IQA)	p-value[¶]	Median (IQA)	p-value[¶]
	Baseline		2 (2)		1 (2)		1 (2)	
Frequency, MHz	6	(*versus* 4)	1 (2)	0.033	1 (2)	0.482	1 (2)	0.010
	8		1 (2)	<0.001[+]	0 (2)	<0.001[+]	1 (2)	<0.001
Gain, %	90	(*versus* 80)	2 (3)	0.030	1 (2)	0.111	2 (2)	0.003
	100		2 (3)	<0.001[+]	2 (2)	0.030	2 (2)	<0.001
Power, dB	−20	(*versus* −10)	0 (0)	<0.001[+]	0 (0)	<0.001[+]	0 (1)	<0.001
	0		2 (2)	0.244	0 (1)	<0.001[+]	1 (2)	0.017
Dynamic range, dB	36	(*versus* 60)	0 (0)	<0.001[+]	0 (0)	<0.001[+]	0 (0)	<0.001[+]
	84		2 (3)	0.154	2 (2)	0.166	2 (2)	0.046
	102		2 (2)	0.207	2 (2)	0.440	2 (2)	0.078
Artefact rejection, elements	32	(*versus* 0)	1 (2)	NS	1 (2)	0.207	1 (2)	0.253
	64		1 (3)	0.009	0 (2)	<0.001[+]	1 (2)	<0.001[+]
Speckle reduction	Level 2	(*versus* off)	2 (2)	NS	1 (3)	NS	1 (2)	NS
	Level 5[#]		1 (1)		1 (2)	NS	1 (1)	NS
	Level 8[#]		1 (1)		1 (2)	0.331	1 (2)	0.042
Frame averaging	2	(*versus* 0)	1 (2)	NS	1 (2)	NS	1 (2)	NS
	4		1 (1)	0.638	1 (2)	NS	1 (1)	0.534
	8		1 (2)	<0.001[+]	1 (2)	0.011	1 (2)	<0.001[+]
Image enhancement	Method 1	(*versus* off)	1 (2)	NS	1 (2)	NS	1 (2)	NS
	Method 2		2 (2)		1 (2)		1 (1)	
	Method 4		2 (2)		1 (2)		1 (2)	

IQA: interquartile amplitude; NS: not significant (pairwise comparisons were not tested). [#]: Data are missing from three patients. [¶]: Pairwise comparisons using significance adjusted to the number of conditions in each parameter. [+]: p-values <0.001 are of uncertain magnitude.

and pressure-regulated ventilation. This approach was integrated with the novel use of the M-mode to target pleura and perceive thorax movements (such as probe movements and spontaneous breaths), including inspiration and expiration. The baseline US recordings showed a higher proportion of VAs during inspiration than during expiration. This makes statistical differences between the baseline and test recordings more likely during inspiration but also suggests that some alveoli collapse during expiration [19]. Additionally, our baseline recordings were made with US settings known not to obscure VAs, avoiding useless comparisons [10–12].

The clinicians in our panel used a qualitative and subjective method to identify VAs during respiration. Despite only a moderate to good intra-observer agreement achieved in recognising and counting VAs [20–22], the human eye interpretation of LUS would always be complemented with desirable LUS quantitative tools [23, 24]. Therefore, we counterbalanced this caveat by increasing the sample size, controlling operator and physiological factors contributing to VAs, implementing the analysis of US cine loops blind to patient details and also blind review of the "extreme" outlier classifications by other experts.

Frequency, signal length and intensity

The signal intensity and length of VAs are known to vary according to the trap configuration and content due to different attenuation coefficients [25, 26]. In our study, the use of an 8 MHz probe frequency consistently decreased the number of VAs, which is supported by other *in vitro* and *in vivo* reports [13, 14, 16, 25–28]. When using high frequencies during LUS, VA length shortens and signal intensity diminishes. This finding is related to the acoustic trap and is reported as more characteristic within the presence of a high cellularity oedema [16].

Other US settings that are known to influence VA signal intensity are gain and system output power [11, 12]. In our study, test conditions of gain improved significantly the number of VAs. Duggan *et al.* [14], in an *in vivo* observational study, found that B-lines were better visualised at higher gains using a curvilinear probe. However, when using gain near the upper limit of the US system, the imaging spatial resolution decrease due to the imaging brightness. In fact, it also increases the width and blurring of the margins of the VAs, and our group suggested adjusting the gain to achieve a hyperechoic signal of the

pleura line without excessive distortion (gain 100%; figure 1a). The US system output is known to relate linearly with B-line signal intensity; therefore, reducing it may potentiate the dismissal of VAs (figure 1a). On the other hand, selecting the upper limit of the US system output power may lead to a higher mechanical index, leading to patient safety concerns [9].

Dynamic range, as expected, also influenced the number of VAs. The range of echoes displayed on the US imaging greyscale is selected by the dynamic range. Low dynamic range (*i.e.* 36 dB) showed a drastic disappearance of VAs, which should be avoided as reported by others [29], whereas allowing a high range of grey colour produces brighter images, increasing the VA signal intensity. Moreover, it also produces images with less contrast between the VAs and the background medium [12, 29]. In this study, a value of 84 dB (*versus* 60 dB) showed a tendency to improve VA detection that was not consistently demonstrated when evaluating individual respiratory cycle phases. Therefore, an intermediate value between 60 dB and 84 dB is advisable, according to our data.

Artefact filtering and image enhancement

In our study, artefact reduction was found to reduce the number of VAs when applied to the 64 probe elements. The US system applies a filter on the majority of the echo signal retrieved by the probe elements. A bandpass filter selects the level of echoes to display and eliminates the remaining ones, considering them as noise [30]. In our data, there was only a decrease in signal intensity after applying level 8 (*versus* off), as shown in figure 1c. Interestingly, despite being nonsignificant, VA signal intensity below the pleura was reduced with the sparing of the VA signal at depth. In fact, another type of filter that has a specific task is the speckle reduction filter, which is used to reduce the graininess of the image and mitigate scattering [31]. The efficacy of using convolutional neural networks to reduce scattering was previously reported by our group [32], supporting our results. Whether a different configuration of acoustic traps is more prone to a specific speckle reduction filter remains unknown. Hence, to improve VA detection, the artefact rejection and speckle reduction filter should be turned off.

Frame average is a tool used to improve imaging details by averaging multiple pixels and displaying their signal means [30]. When using level 8 of frame averaging, the US imaging showed a significant delay during lung sliding. This effect consistently blurred and decreased the VA number (supplementary video 1 and supplementary material). Although their impact was easily discernible on clinical practice cine loops, it may be absent on motionless frames. In our previous laboratory studies [12] frame average (at level 2) increased the quality of the VAs by improving the signal intensity in far depth. To some extent, the influential effect of this US setting was distinctive in *in vivo* experiments, reassuring the need for *in vivo* testing.

In our study, we also selected some experimental methods for image enhancement. Either by applying a smooth filter to each pixel signal, averaging the largest signals and filtering out the lowest ones, or by the amplification of more-intense signals (method 1, method 2 and method 4, respectively) [30, 31]. Although the theoretical usefulness of this tools to improve VA signals, statistical differences between test conditions and baseline recordings were not identified. It remains to be studied if US image enhancement processing algorithms can modulate and extract useful data about VA origin.

Limitations

Nowadays, more VAs are recognised than before the SARS-CoV-2 pandemic. The patients showed VAs with different aetiologies, which may have an impact on the magnitude of the tested setting [33, 34]. Moreover, the acoustic trap configuration should be intrinsically different across diseases (*i.e.* interstitial pulmonary oedema *versus* cardiogenic pulmonary oedema) [35–38]. Therefore, US settings may have a different impact on other types of VAs not evaluated in this study, which remain to be studied. Moreover, the counting of VAs remains subjective in clinical practice despite the development of automatic methods for VA detection [23]. The existence of reports with excellent intra-observer agreements when counting and identifying VA abnormalities [22], interpreting their origin is still poorly reproducible between centres. The latter may be a sign of the urgent need for LUS standardisation [8, 9, 34]. Nonetheless, our study may encourage the improvement of algorithms by suggesting US pre-set adjustments.

The ICU environment is also hazardous for immobile probe recordings, which led to the exclusion of some US cine loops. Practice recordings were done by using a gyroscope mounted in a secure metal support, gentle tilting or rocking movements to exclude their systematic occurrence [12].

Conclusion

In this *in vivo* study, the tested US settings showed a mild influence on the VA number after controlling for physiological and operator LUS confounders. To avoid a negative impact of US setting on VA, the

system pre-set should use a lower probe frequency, with a gain and power adjusted for a value near the available upper limit with a hyperechoic pleura (avoiding excessive brightness), an intermediate value of dynamic range adjusted for a discernible background medium contrast, without any other artefact filtering nor image enhancement tools.

Provenance: Submitted article, peer reviewed.

Data availability: The research team provided supplementary data describing the setup used for data acquisition (supplementary figure 1) and a demonstrative LUS recording from a patient showing the test recordings (supplementary video 1). Additionally, the ultrasound pre-sets used for the test recordings are provided, as well as the nonparametric test results and individual patients data (*i.e.* demographic, clinical, respiratory and renal data). The imaging dataset is available on request.

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Ethics statement: The Ethics Committee of the Hospital Garcia de Orta EPE (Almada, Portugal) approved the study protocol (number 49/2023). Subjects were asked to sign a digital informed consent form before IMV or after IMV weaning. In the case of cognitive impairment in both periods, informed consent was sought from the patient's legal representative. If not available, the Ethical Committee was contacted and the need for informed consent was waived.

Author contributions: J. Leote and H. Dias made substantial contributions to the conception of the project; the acquisition and analysis; and the interpretation of data for the work. A. Gonçalves and J. Fonseca drafted the work. F. Gonzalez and J. Leote recruited patients and performed the evaluation protocol during the study. R. Loução, F. Gonzalez and J. Bacariza revised the study conception and the manuscript critically for important intellectual content. All authors provided final approval of the version to be published.

Conflict of interest: The authors declare that they have no competing interests. This research did not receive any funding.

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